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# **A COMPARISON OF STRESS RESPONSES IN SEA URCHINS AND SEA CUCUMBERS EXPOSED TO SALINITY AND HANDLING STRESS**



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# Introduction



- Sea urchins are echinoderms that are generating interest in aquaculture
- Sea urchins are particularly valuable as:
  - Research models
  - Delicacy food item
- Many sea urchin populations have been greatly overfished
  - Depletion of European and Asian stocks has reduced sea urchin quality



Photo by Navid Ayon

# Introduction



- **Animals can be raised in aquaculture conditions**
  - Prevents:
    - ✦ Overfishing
    - ✦ Pollutant contamination
    - ✦ Loss to predation
    - ✦ Low quality product
    - ✦ Damage to ecosystem
- **However, organisms encounter stress in intensive aquaculture environments**
  - ✦ Some species are hardier than others
  - ✦ Hardy species make better aquaculture candidates
    - More resistant to disease
    - Better production
    - Easier to raise

# Research Objectives



- To compare the stress responses of echinoderms in conditions that might be encountered in an aquaculture environment.
  - Handling
    - ✦ Animals are moved from tank to tank
    - ✦ In research facilities, they must be handled for sampling
  - Salinity change
    - ✦ Rain water can dilute salinity of culture ponds
    - ✦ Purchasing ocean mimicking salt mixes is expensive



Photo by Navid Ayon

# Materials and Methods



- Adult purple sea urchins (*Stronglyocentrotus purpuratus*) and giant California sea cucumbers (*Parastichopus californicus*) were obtained from Bodega Marine Laboratory Station in Bodega Bay, California.
- Three treatment groups were established
  - Handling
    - ✦ Three times daily for 5 minutes; kept at optimal salinity (34 ppt)
  - Low salinity
    - ✦ Kept at 28 ppt
  - Controls
    - ✦ Optimal salinity (34 ppt) and never handled
- After 72 hours in treatment conditions, coelomic fluid was collected for analyses of cells (coelomocytes)

# Materials and Methods



- Coelomocytes were counted via hemocytometer for differential and total coelomocyte count (Braak, 2002)
- Total protein content was read via protein refractometer (Mustafa et. al. 2000)
- Lytic activity was determined by lysozyme turbidity assay (Chia & Xing, 1996)



Photo by Tazin Fahmi

# Material and Methods



- Phagocytic capacity of cells was determined by counting cells with engulfed bacteria (formalin-killed) bacteria (Mustafa et. al. 2000)
- Respiratory burst activity was determined by spectrophotometer

$$\text{Phagocytic capacity} = \frac{\text{Number of cells with engulfed bacteria}}{\text{Total number of cells}} \times 100$$

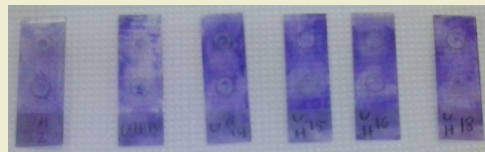


Photo by Stacy Keough



# Coelomocyte Studies

- Changes in coelomocytes
  - ✦ Total and differential sea urchin cell count
    - ✦ Phagocytic
    - ✦ White spherule
    - ✦ Red spherule
    - ✦ Vibratile
  - ✦ Coelomic fluid protein
  - ✦ Phagocytic capacity
  - ✦ Lytic activity

(Smith et. al. 2010)

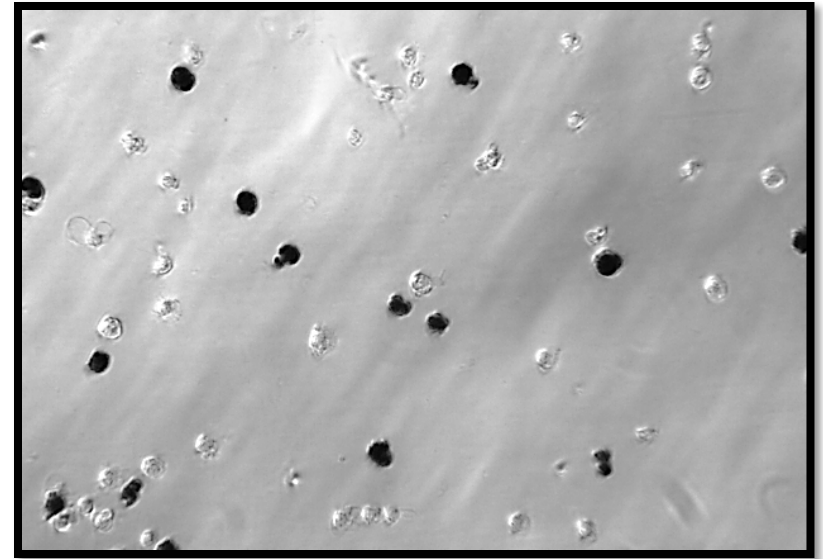


Photo by Regina Shannon

Sea Urchin Cells

# Types of Sea Urchin Cells

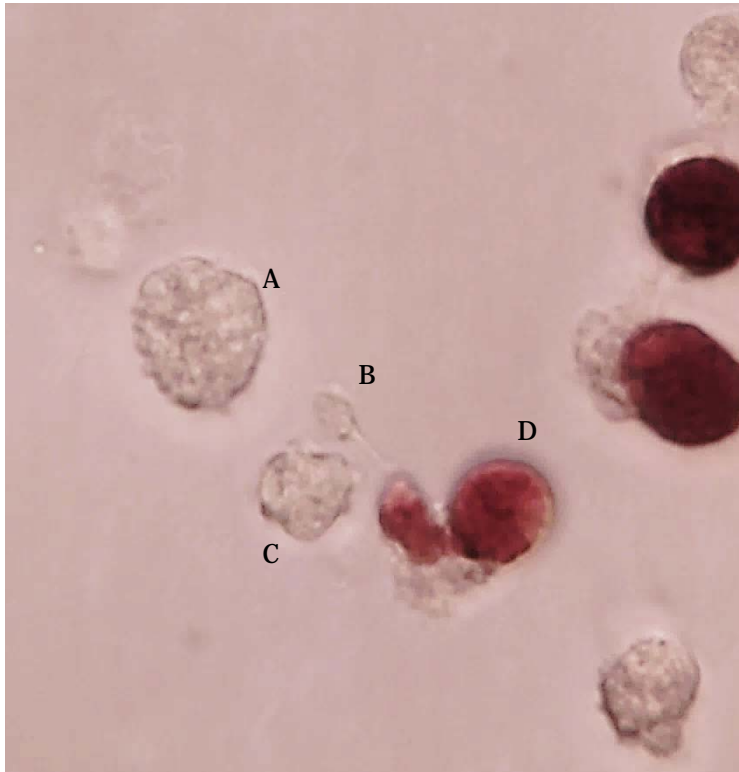
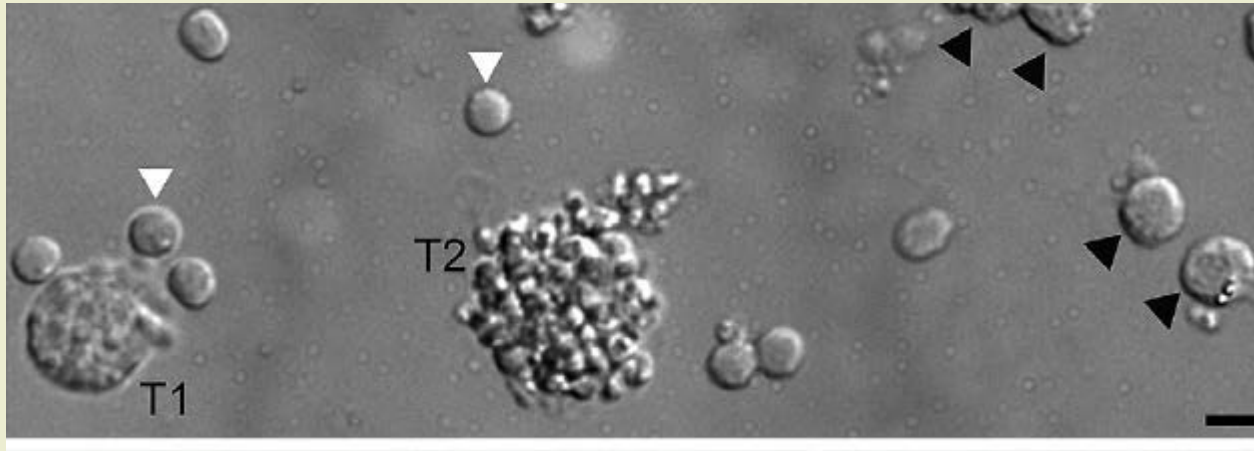


Photo by Regina Shannon

**Sea urchin cell types**

- A—White spherule cell
- B—Vibratile cell
- C—Phagocytic cell
- D—Red spherule cell

# Types of Sea Cucumber Cells



Sea cucumber cell types

A—Lymphocyte

B—Type 1 and Type 2 spherule cell

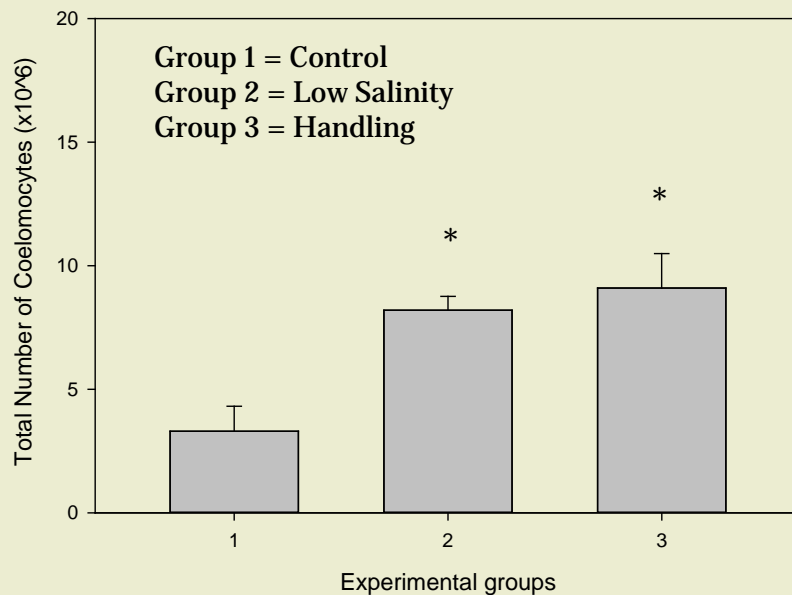
C—Phagocytic cell

# Results: Total Coelomocyte Count

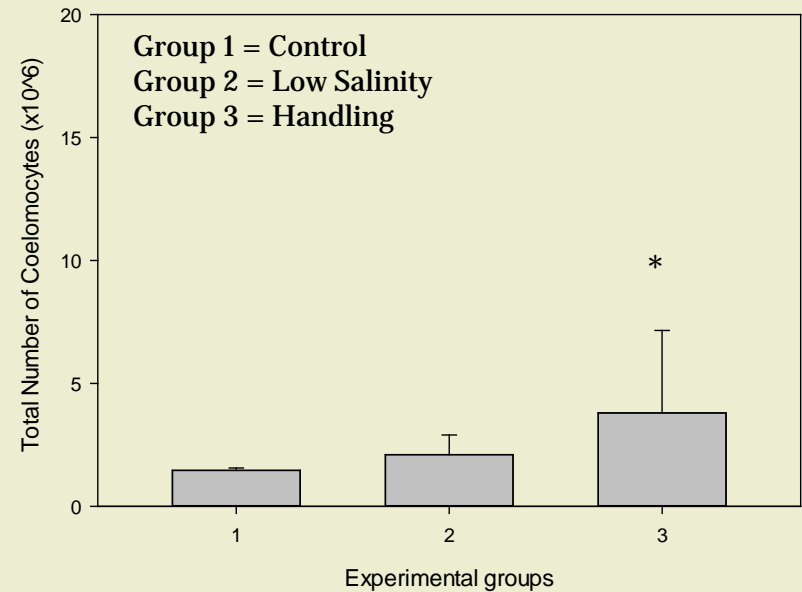


## Total Coelomocyte Count

### Sea Urchin



### Sea Cucumber

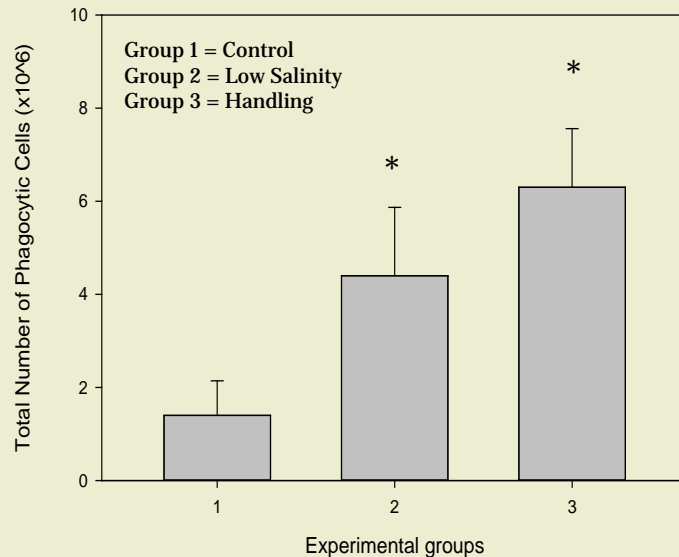


# Results: Differential Cell Counts

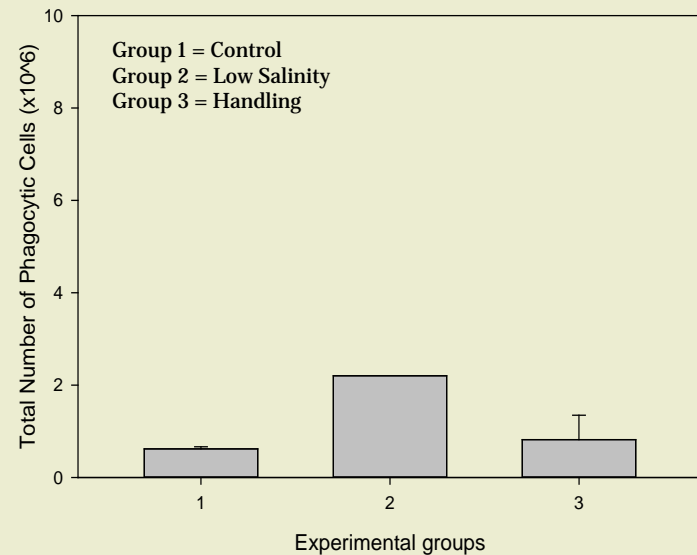


## Number of Phagocytic Cells

### Sea Urchin



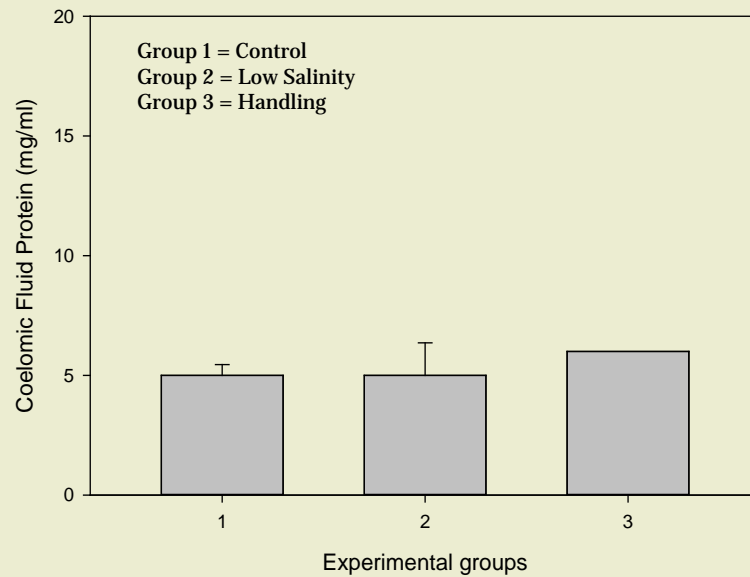
### Sea Cucumber



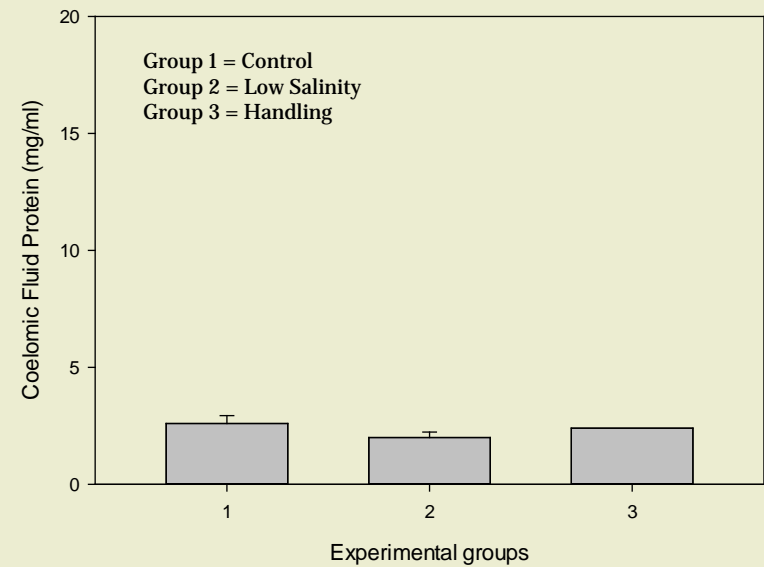
# Results: Coelomic Fluid Protein



## Sea Urchin



## Sea Cucumber

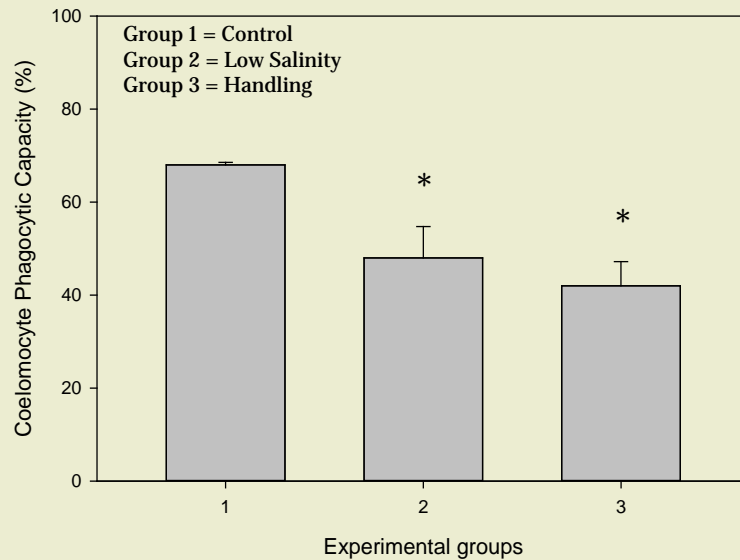


# Results: Phagocytic Capacity

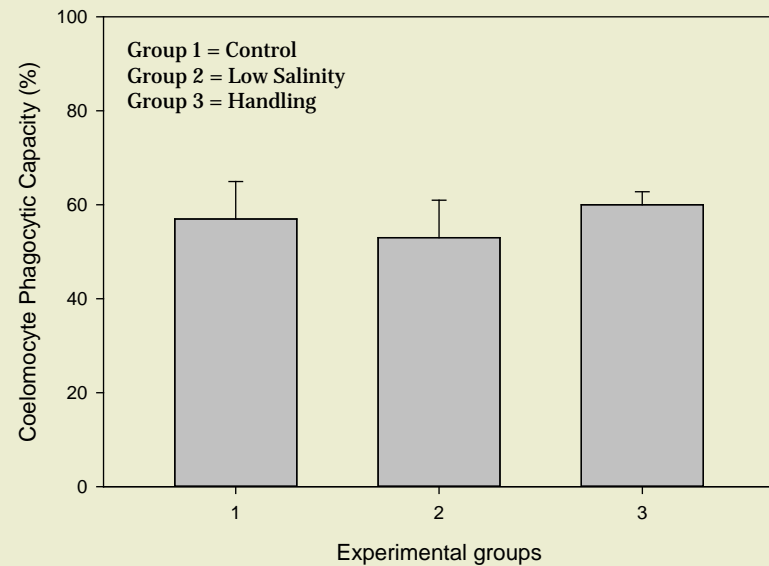


## Phagocytic Capacity of Phagocytic Cells

### Sea Urchin



### Sea Cucumber

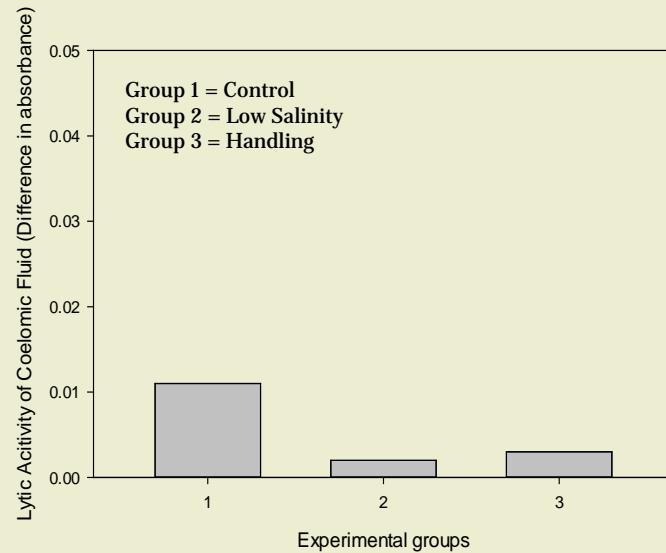


# Results: Lytic Activity

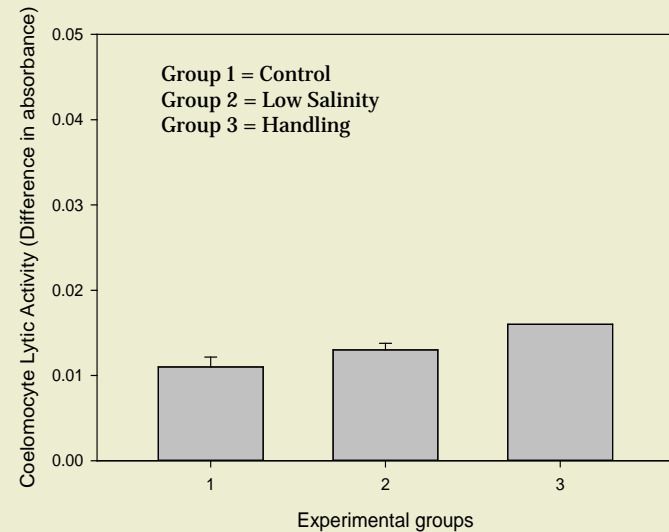


## Lytic Activity of Coelomic Fluid

### Sea Urchin

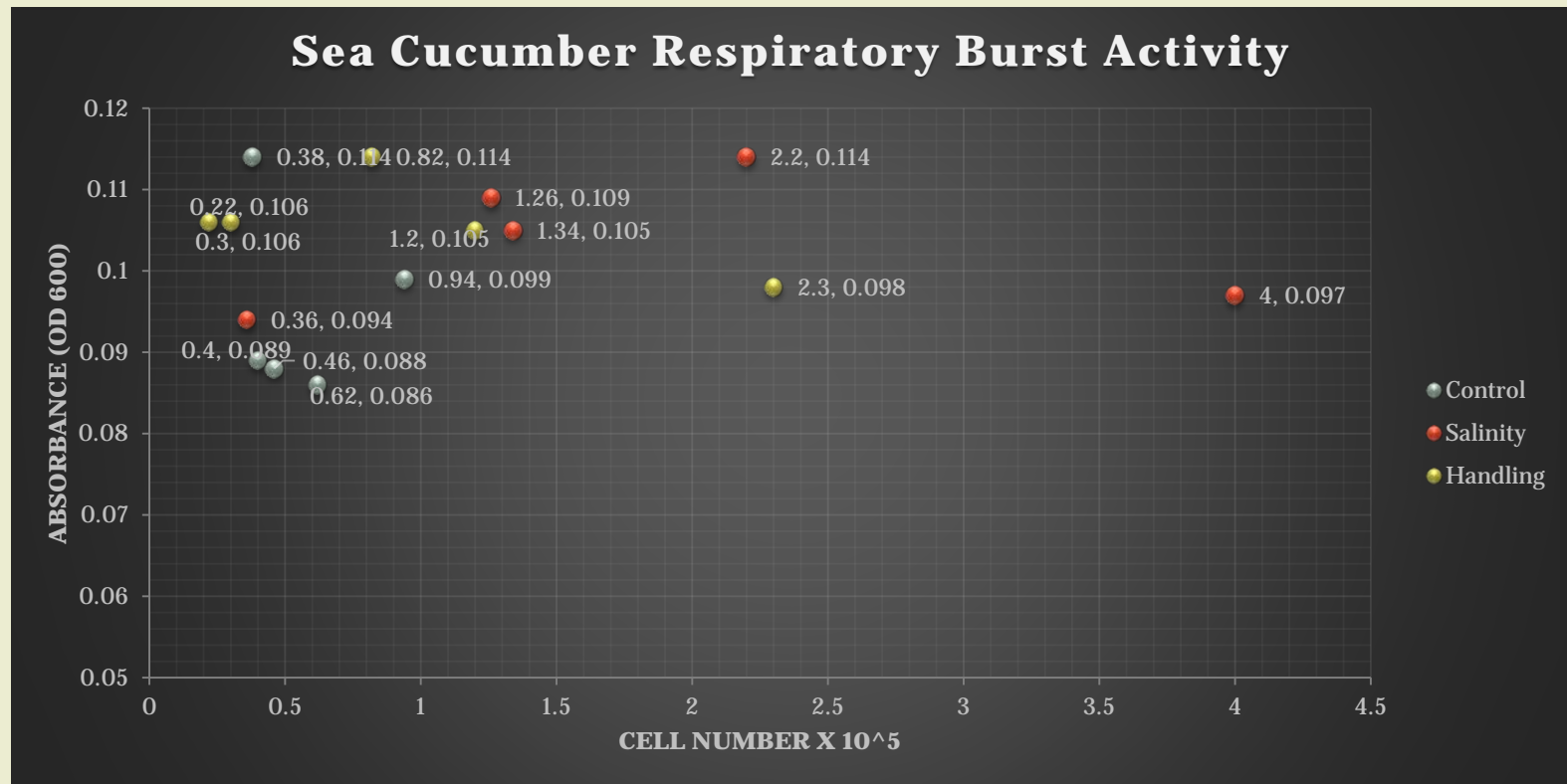


### Sea Cucumber

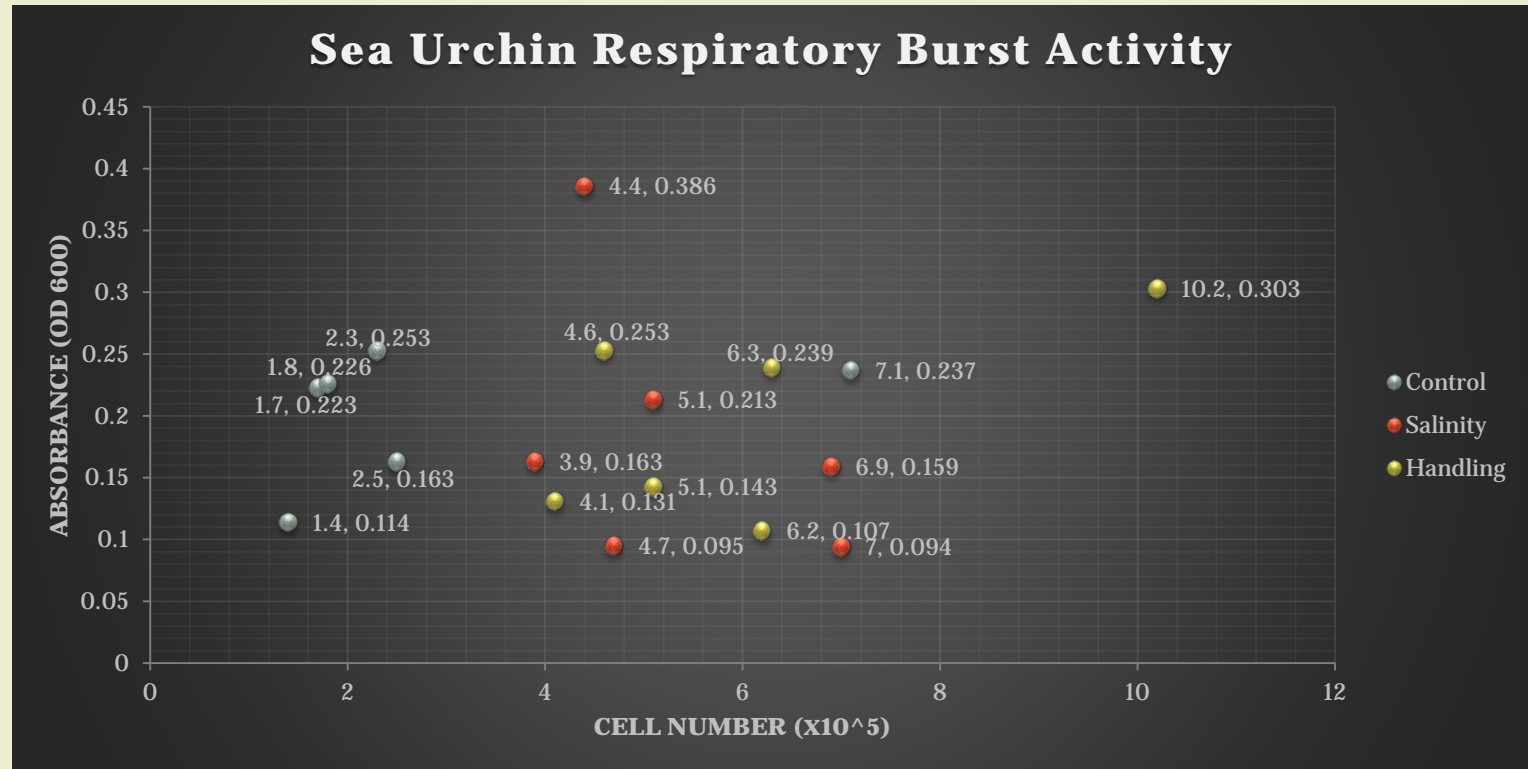




# Results: Respiratory Burst Activity



# Results: Respiratory Burst Activity



# Conclusion



- It appears that both handling and low salinity produce significant stress responses in sea urchins, though not significant stress in sea cucumbers.
- This would indicate that sea cucumbers are a hardier aquaculture candidate than sea urchins.



Photo by Stephen Shannon

# Impact



- Our study of the physiological and immunological parameters in invertebrate aquaculture can be used for increased production and to make better pharmaceuticals in the future.
- An increase in culturing these species will help reduce the risk of overfishing.
- Intensive aquaculture will prevent problems associated with off-shore culturing.

# Further Research



- **Areas for further research include:**
  - Effects of varying salinities on animal health
  - Longer term study
  - Studies utilizing the impact of nutraceuticals on animal immune function



Photo by Tazin Fahmi

# Acknowledgments



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# Questions?



## production



In stress testing, the giant California sea urchin (left) has much lower levels of response than those found in the graph sea urchin.

## Stress Susceptibility Of Echinoderms Under Aquaculture Conditions

### Summary:

If an animal is stressed, its immune function decreases, and vulnerability to disease increases. Since there is currently no effective treatment for disease in echinoderms, the authors examined the impacts of handling and salinity fluctuations on the immune function of sea urchins and sea anemones. The sea urchins were much more susceptible to stress and decreased immune function due to limited salinity and handling than the sea anemones were. Although sea anemones did show negative effects, none of them was significant.

Invertebrates such as sea urchins and sea anemones have been widely researched throughout Asia for ornamental and pet keeping. Importation as a source of both high-quality pets and valuable pharmaceuticals has increased over the past few years. They are sources of both high-quality pets and valuable pharmaceuticals, but echinoderms have been found to have a variety of environmental and physiological problems, as well as reproductive problems and pharmaceutical value. The authors have long been used as a research model and a food source since ancient antiquity.

Due to their various factors, their animal populations have become the source of a growing fishery system. How-

ever, as is commonly found in fisheries, there is evidence that the invertebrates may become overexploited, which leads to population collapse. In giant California sea urchins have recently become an important export from the United States to various countries, especially Japan. It is essential that measures be taken to prevent that overexploitation, while at the same time growing and sustaining the industry. One way to establish aquaculture facilities that are sustainable over time is to increase the immune function of the giant California sea urchins, *Paracentrotus giganteus*, and the purple sea urchin, *Diadema setaceum*, particularly sea urchins.

**Stress Response** Although immune responses can protect against disease, they are also valuable in a variety of ways. These include wound healing, handling, and salinity and temperature fluctuations.

If an animal is stressed, its immune function decreases, and vulnerability to disease increases. There is currently no effective treatment for disease in echinoderms, so the use of echinoderms can lead to the development of disease-resistant lines that are more desirable for aquaculture.

**Stress Study** The authors examined handling and salinity stress on sea urchins and sea anemones under

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The authors examined the immune function of the giant California sea urchin, *Paracentrotus giganteus*, and the purple sea urchin, *Diadema setaceum*, particularly sea urchins.

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**Experimental Setup** Sea urchins and sea anemones were

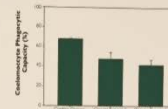


Figure 1. Phagocytic activity of sea urchin phagocyte cells.

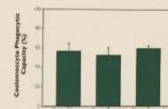


Figure 2. Phagocytic activity of sea urchin phagocyte cells.

### Perspectives

Based on the results obtained in the study, it appeared that sea urchins were more susceptible to representing stress and decreased immune function due to limited salinity and handling than the sea anemones were. This would indicate that the sea urchins were more susceptible to stress and handling than the sea anemones were.

Interestingly, although sea urchins did show more negative effects from low salinity and handling, none of them was significant. This could indicate a genetic degree of resistance to salinity changes and handling, which would make them a good candidate for aquaculture.

A follow-up study could explore sea urchins in fresh conditions conditions such as decreased pH conditions, but a decreased effect. In addition, studies addressing immunosuppression as a result of salinity stress could be done to see if there is a greater effect on the immune function of echinoderms exposed to stressful conditions.

obtained from Biology Marine Laboratory, Marion, Indiana, U.S.A., and acclimated prior to the study. They were divided into three groups with one replicate per group.

Control groups were kept in the original salinity of 30 ppt and were handled. The handling-repeated groups were kept at 30 ppt and handled daily, while the low-salinity-repeated groups were kept at 20 ppt and were handled. Temperature



A sea urchin under stress in a petri dish. The sea urchin was exposed to low salinity and handling.

was maintained at 18°C ± 1, and the pH was maintained at 8.0. The echinoderms were maintained for 72 hours, and then echinoderms from each individual in each of the treatment groups was collected for analysis. Parameters included echinocyte packed cell volume, echinocyte basal protein, and differential echinocyte count, and phagocytic cell activity.

**Results** For the sea urchins, echinocyte packed cell volume and echinocyte basal protein were significantly higher in the control groups, as well as significantly lower in the echinocyte count and echinocyte cell activity.

In contrast, for the sea anemones, the echinocyte packed cell volume and echinocyte basal protein were not significantly different from the control groups, but the echinocyte count and echinocyte cell activity were significantly lower in the echinocyte count and echinocyte cell activity.